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RESIDUES on ALFALFA--

Spray versus granulated materials

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BHC RESIDUES ON ALFALFA— Spray Versus Granulated Materials[1]

GEORGE W. WARE

Technical grade benzene hexachloride (BHC) has probably been the most economical and efficient insecticide used for the control of the meadow spittlebug, *Philaenus leucophthalmus* (L.). It was first recommended for control of the spittlebug on forage crops in Ohio in 1948 and has since been used in Ohio as well as in a number of other states with outstanding success. During this period it was recommended at the rate of 0.25 pound of the gamma isomer per acre.

Weaver and King (1954) and Weaver (1956) obtained equally good control of spittlebug nymphs on alfalfa and red clover using early spring applications of BHC as a spray and as a granulated material at the rate of 0.25 pound gamma isomer per acre. Marshall and Gyrisco (1951) also obtained nearly perfect control on the same host crops using 0.30 pound gamma isomer per acre as a spray formulation.

In view of the successful use of technical BHC for spittlebug control a bioassay residue study of the gamma isomer on alfalfa was undertaken in 1956, using *Aedes aegypti* larvae as the test organism (Burchfield and Hartzell, 1955). The results of this work indicated that on first cutting alfalfa no detectable gamma isomer remained 21 days following application of 0.25 pound gamma isomer per acre in either a spray or a granulated formulation, and none after 14 days on second cutting alfalfa (Osborn, 1957). However, since the gamma isomer is the only one of five predominant isomers in technical BHC considered insecticidal (Metcalf, 1947 and Sherman, 1948), the persistence of the other isomers could not be determined by bioassay.

As a result, in 1957 the residue laboratory was equipped to conduct the Schechter-Hornstein colorimetric method of total BHC analysis (Schechter and Hornstein, 1952), as modified by Wylegala *et al.* (1956).

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[1]Contribution from Regional Project NC-33. Pesticide Residues on or in Food, Feed and Forage Crops (Their Magnitude, Character and Persistence.)

METHODS

On May 8, 1957, duplicate plots of first cutting alfalfa, 75 feet by 13 feet (0.022 acre) in size, separated by 15 foot alleys, were treated with BHC as an emulsion spray and with 30-60 mesh granulated material (2 percent gamma isomer) at two rates: 0.2 and 0.8 pound gamma isomer per acre.[2] Two additional plots were left untreated to serve as checks. The sprays were applied at the rate of six gallons per acre (30 P.S.I.) with a 13 foot conventional boom-type weed sprayer mounted on a Jeep which had been modified for experimental spray purposes. The granulated material was applied with a wheelbarrow-mounted Oliver grass seed drill. (See Fig. 1). At the time of application, the first cutting alfalfa was in the first harvest year and 11 inches in height. Red clover and weeds were abundant.

[2]The emulsifiable concentrate used in spray applications and to make the granulated material was declared by the formulator to be 12 percent gamma isomer. However, the technical material in this concentrate actually contained 40 percent gamma isomer.

Fig. 1.—Experimental applicator made from the grass seed box of a drill used for applying granulated materials.

On June 26, 1957 the second cutting alfalfa at another test location was treated in a like manner. The second cutting alfalfa was four inches in height at application, was in its second harvest year and had a thick stand.

The two percent gamma granulated material was made by spraying one-year-old Attaclay granules in a rotating cement mixer with a xylene solution of the emulsifiable concentrate used in the spray applications.

Sampling: Alfalfa samples were collected by cutting two swaths across each plot with a 30 inch self-propelled sickle-bar mower. The material from the two swaths was collected in large double-lined Kraft paper bags immediately after cutting and placed in a walk-in cooler (1-3 hours) at 40° F. until packed in individual samples for freezing.

Starting with the checks and proceeding from the lower to the higher treatment rates, the alfalfa from both plots of the same treatment was mixed thoroughly on a large work table covered with wrapping paper and packed as well labeled, one-pound samples. The sample containers were 10-pound polyethylene poultry bags.

Three one-pound samples were packed for chemical assay and one larger sample was used for dry weight determinations. Except for the dry weight sample, the samples were held in a walk-in freezer at 0° F. until removed to the residue laboratory. There they were held in a 10 cubic foot deep-freeze, also at 0° F.

Sampling Stripping and Extraction: The samples were removed from the freezer and sliced immediately on a hand-operated bacon slicer. The sliced material was halved and each half minced five minutes on an Omnimixer[3] in a two-quart Mason jar with 400 ml. of glass-distilled Skellysolve "B". The two halves were combined in a one-gallon large mouth jar fitted with an aluminum foil-lined lid and placed on the roller for one hour. After rolling, the samples were filtered into six inch crystallizing dishes and allowed to drain for 15 minutes.

The dishes were then placed in the hood and evaporated to dryness (3-4 hours). Following evaporation the residues were taken up with 150 ml. of glass-distilled methylene chloride in three washings and transferred to one-pint prescription bottles. To the methylene chloride solutions were added 25 ml. volumes of acetone and five grams of vegetable carbon. The bottles were capped and shaken for three minutes and the contents filtered. This was followed by washing the filters with two 50 ml. portions of methylene chloride. This carbon treatment removed all interfering materials. The sample extracts were held in the deep-freeze at 0° F. for analysis.

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[3]An all purpose high speed mixer, manufactured by Ivan Sorvall, Inc., Norwalk, Conn.

Standard Curve: In order to interpret the optical density readings of the alfalfa residues three standard curves were run using technical grade BHC (40 percent gamma isomer)[4] added at different stages during stripping of the untreated, one-pound alfalfa samples. The standard curves were derived from three points of BHC fortifications: (1) fresh alfalfa before freezing, (2) frozen alfalfa after hand-slicing, and (3) the solvent following mincing. Not all of the resulting standard curves were identical, as might be expected. Recoveries from fortifying at locations (2) and (3) were similar while that fortified before freezing was consistently lower. (Figure 9).

Analytical Method: The colorimetric BHC analysis method used here follows that described by Wylegala *et al.* (1956), except for the composition of the nitrating acid, purification of malonic acid, and use of phosphoric acid as the glass-fittings lubricant. The above method calls for a nitrating acid made 1:1 (vol/vol) of concentrated sulfuric and fuming nitric acids. Consistent results could not be obtained in this study until a nitrating acid of 20:1, sulphuric to fuming nitric, was used as suggested by Hancock and Laws (1955). It is believed that water in small quantities remained in the sample extract and that this water was vaporized into the nitrating tube either reducing the effectiveness of the nitrating acid or resulting in the conversion of meta-dinitrobenzene to some other compound.

It was observed that Eastman Organic Chemicals' practical grade malonic acid produced a blank as low as that of malonic acid recrystallized from ethyl acetate, suggested by Gunther and Blinn (1955).

Glacial acetic acid served as the glass-fittings lubricant suggested by Gehrke and Bevirt (1956) and Gehrke *et al.* (1957). The authors consistently obtained lower lindane readings when the joints were lubricated with 85 percent phosphoric than with acetic acid. However, Wylegala *et al.* (1956) and Fahey and Rusk (1958) experienced no difficulty when phosphoric acid was used.

The entire sample extract, or an aliquot when high levels of BHC were anticipated, was placed in a 250 ml. balloon flask and the solvent removed on an 18 inch Vigreaux column. When the extract was reduced to approximately 25 ml., 125 ml. of glacial acetic acid was added through the thermometer coupling. The temperature of the column was allowed to reach 100° C., the condenser stopcock was closed, and 50 ml. of acid was collected. The heating mantle was then removed and the apparatus was exhausted with CO_2 to avoid contamination by atmospheric benzene and other volatile interfering substances. By distilling

[4]Supplied by United Co-Operatives, Inc., Alliance, Ohio.

over 50 ml. of acetic acid, all volatile materials including water, with boiling points lower than acetic acid are theoretically removed from the test sample extract. By using this Vigreaux column distillation system it was not necessary to purify the glacial acetic acid as suggested in the original Schechter-Hornstein method and by Klein (1956).

After cooling the extract to room temperature and adding two grams of malonic acid and five grams of zinc powder the flask was attached to the nitration apparatus. The mantle voltage was advanced gradually for 1½ hours until refluxing began. Refluxing was continued for 1½ hours.

Following the nitration period the nitrating column was removed and drained into a 60 ml. separatory funnel and processed with 2-butanone and 40 percent aqueous potassium hydroxide to produce the violet color characteristic of meta-dinitrobenzene in the presence of strong alkali. The butanone-KOH solution was then shaken in darkness for 20 minutes on a mechanical shaker. Optical density of the 2-butanone layer was read immediately on a Bausch and Lomb "Spectronic 20" at 565 mμ using the standard half-inch cuvette and 2-butanone as a reference blank. Untreated checks invariably produced readings as low as or lower than the 2-butanone blank.

RESULTS

From Figures 2, 3, 4, and 5, and Tables 1 and 2, it is seen that the initial deposits of respective applications on both first and second cuttings of alfalfa were not the same, probably because of the crop composition and plant height. In the first cutting, which had a heavy proportion of broad-leaf weeds and grass and averaged 11 inches in height, there were many more plant axils and culms in which the granules could lodge, thus leaving a heavier deposit. However, in the first cutting spray applications the lower ratio of leaf surface to weight would result in a lower deposit than on the small plants of the second cutting which had a high leaf surface to weight ratio.

In spray applications the second cutting had the heaviest initial deposits at both concentrations. On the other hand the first cutting had the greatest granular deposits. Spray deposits on second cutting were heavier than on first cutting probably because of the thick stand and small size of the second cutting plants. For the same reason the granulated material on these small plants apparently fell through to the soil.

At the time of harvest the first cutting of alfalfa showed heavier BHC residues on the plots treated with granulated material than on those sprayed. This can be explained by the fact that BHC is translocated in

alfalfa when treated with granulated material (Osborn, 1957). The site of initial deposit of the two formulations undoubtedly plays the biggest role in this harvest residue phenomenon, i.e., spray on foliage, granulated material on the soil.

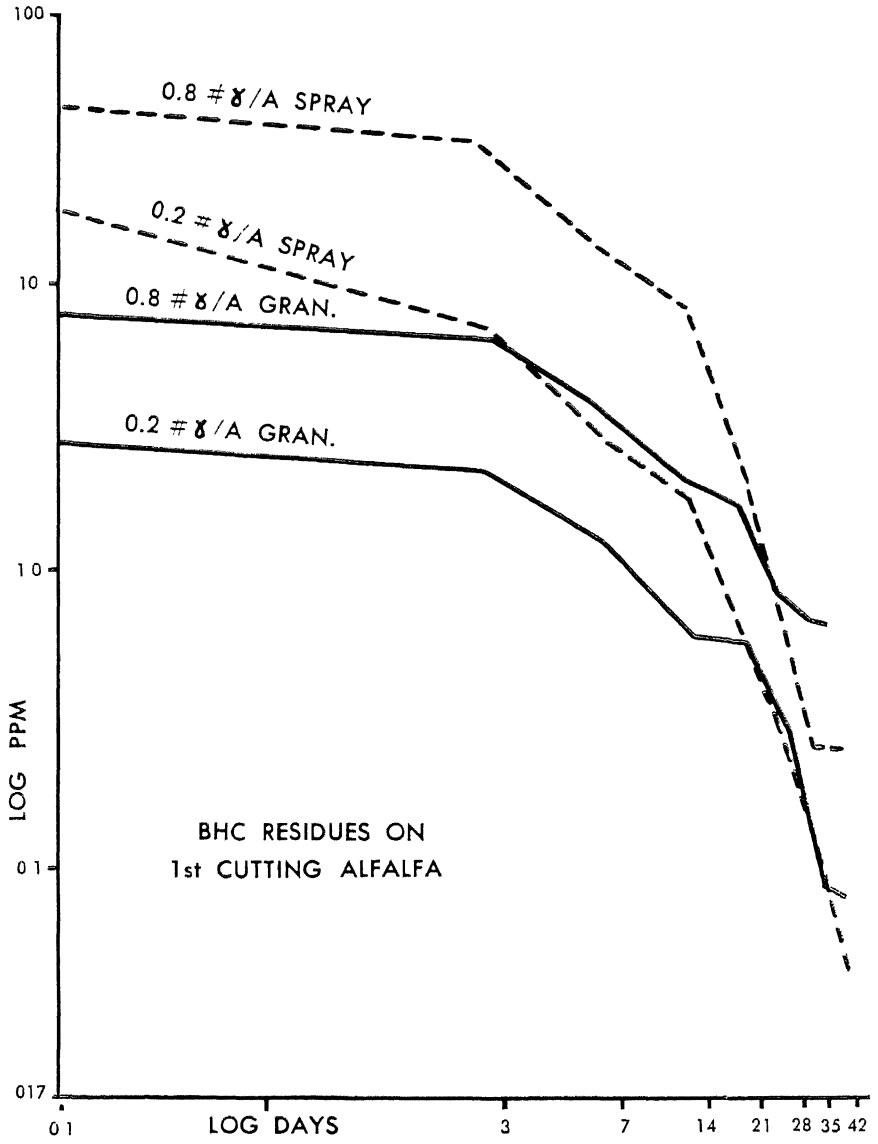

Fig. 2.—Degradation curves of BHC on first cutting alfalfa resulting from spray and granular applications.

It was observed during application of sprays and granulated materials to alfalfa, that the sprays thoroughly wetted the plants with little or no run-off, depending on crop height. However, with granu-

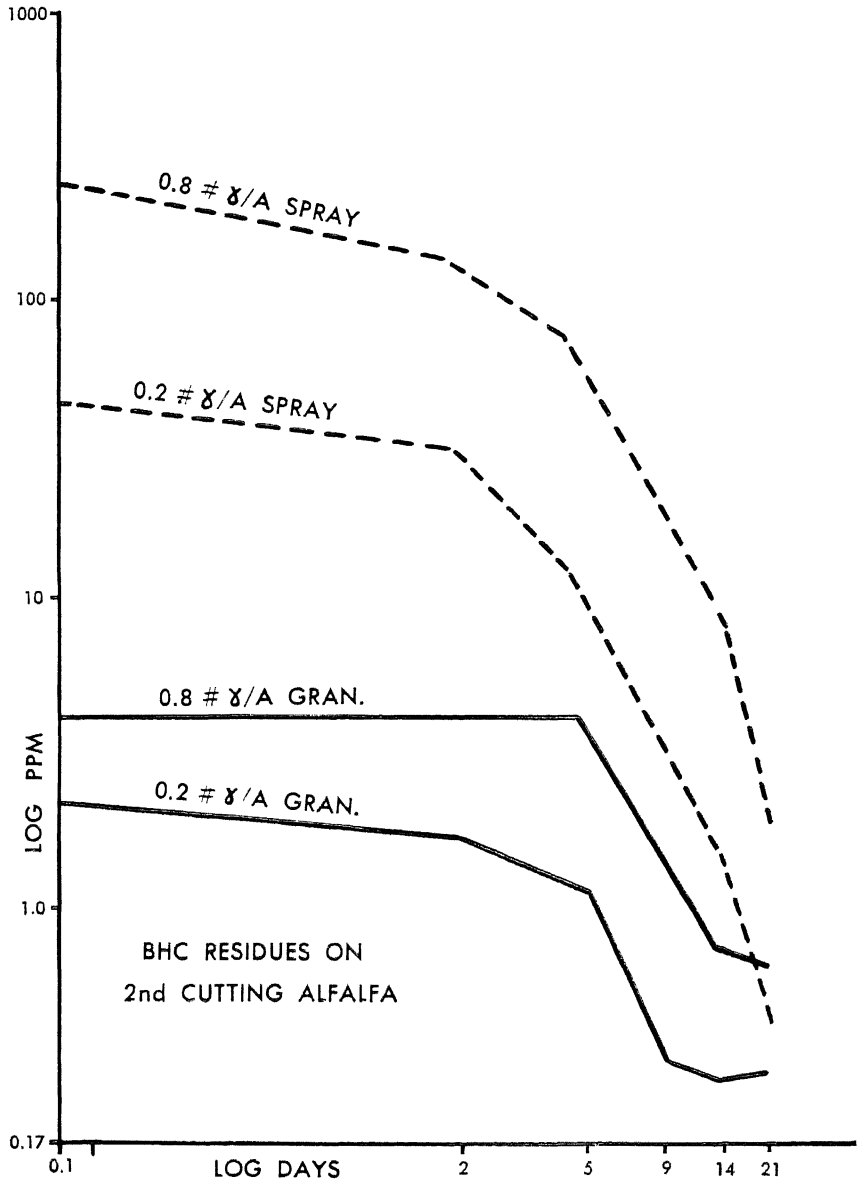

Fig. 3.—Degradation curves of BHC on second cutting alfalfa resulting from spray and granular applications.

lated materials on first cutting it was estimated that 75 percent of the granules fell through the foliage to the soil. On second cutting alfalfa, when the stand was short and dense, but contained very little grass and weeds, an estimated 90 percent of the granules were found on the soil immediately following application.

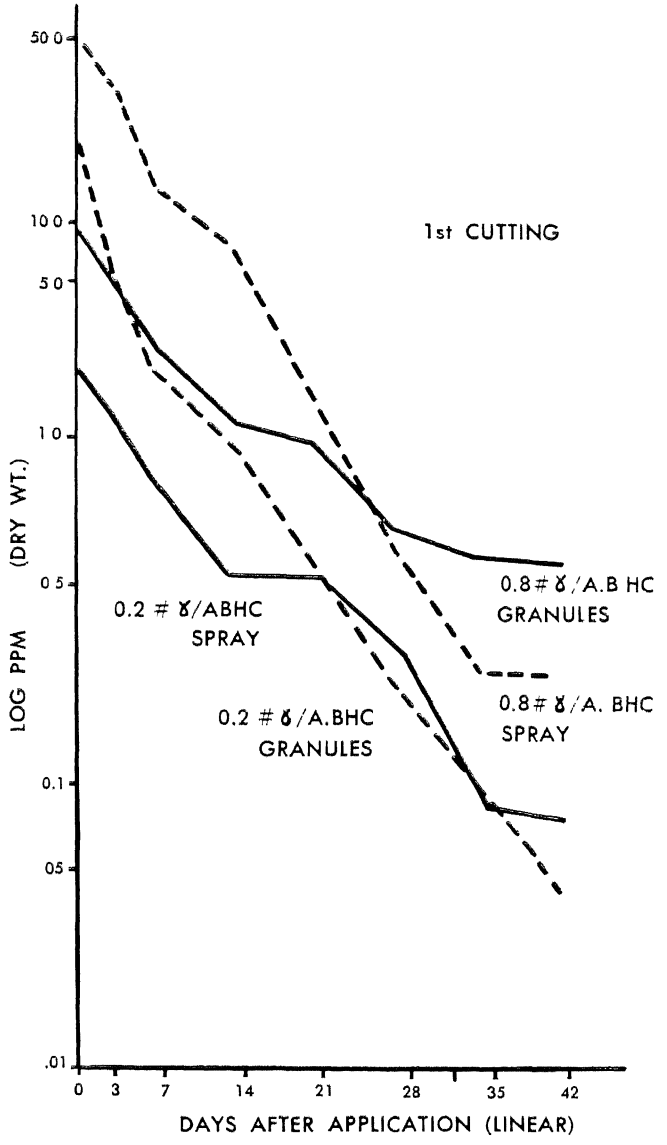

Fig. 4.—Semi-log degradation curves of BHC on first cutting alfalfa resulting from spray and granular applications.

It will be seen in Figures 3 and 5 that the granulated material residue slopes on second cutting for the 21 day sampling period strongly resemble the corresponding slopes at this period on first cutting shown in Figures 2 and 4.

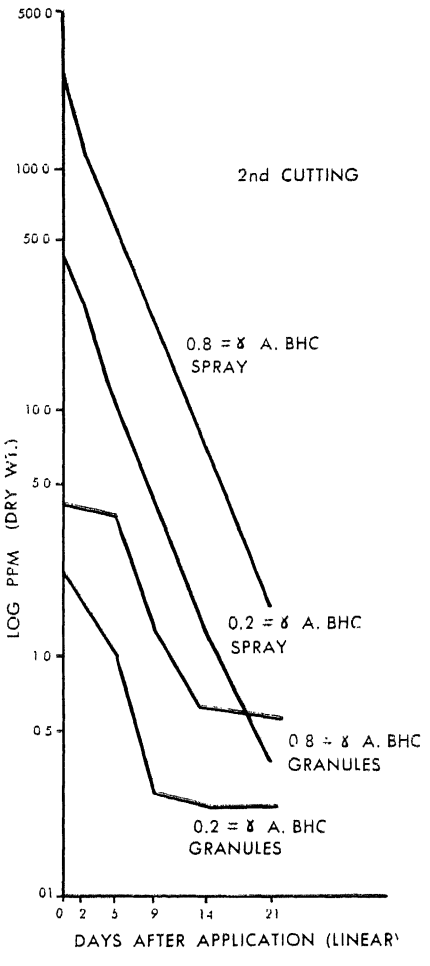

Fig. 5.—Semi-log degradation curves of BHC on second cutting alfalfa resulting from spray and granular applications.

Figures 7 and 8 show that the regression slopes of the first and second cuttings established by the method of least squares are quite consistent, indicating that the rate of disappearance for the two cuttings is approximately the same. Considerable differences however, will be observed between the slopes of granulated material and spray applications.

DISCUSSION

Ginsburg *et al.* (1952) sprayed alfalfa with 4.0 pounds per acre of crude BHC wettable powder, obtaining a residue at five days of 21.6 ppm (wet weight), whereas the amount of crude BHC sprayed in the present study (40 percent gamma) would be 0.5 and 2.0 pounds per acre, resulting in wet weight residues of 2.3 and 14.4 ppm for the same time interval.

Hopkins *et al.* (1952) dusted alfalfa with lindane and BHC at the rate of 2.0 pounds actual toxicant per acre. It is believed, from the residue data presented, that the BHC was technical grade, applied on the basis of gamma isomer content. The lindane dust at the end of 42 days showed a residue of 0.1 ppm while the BHC showed a residue of 0.62 ppm, wet weight. A graphic comparison of the data of this work and those of the above two workers appears in Figure 6.

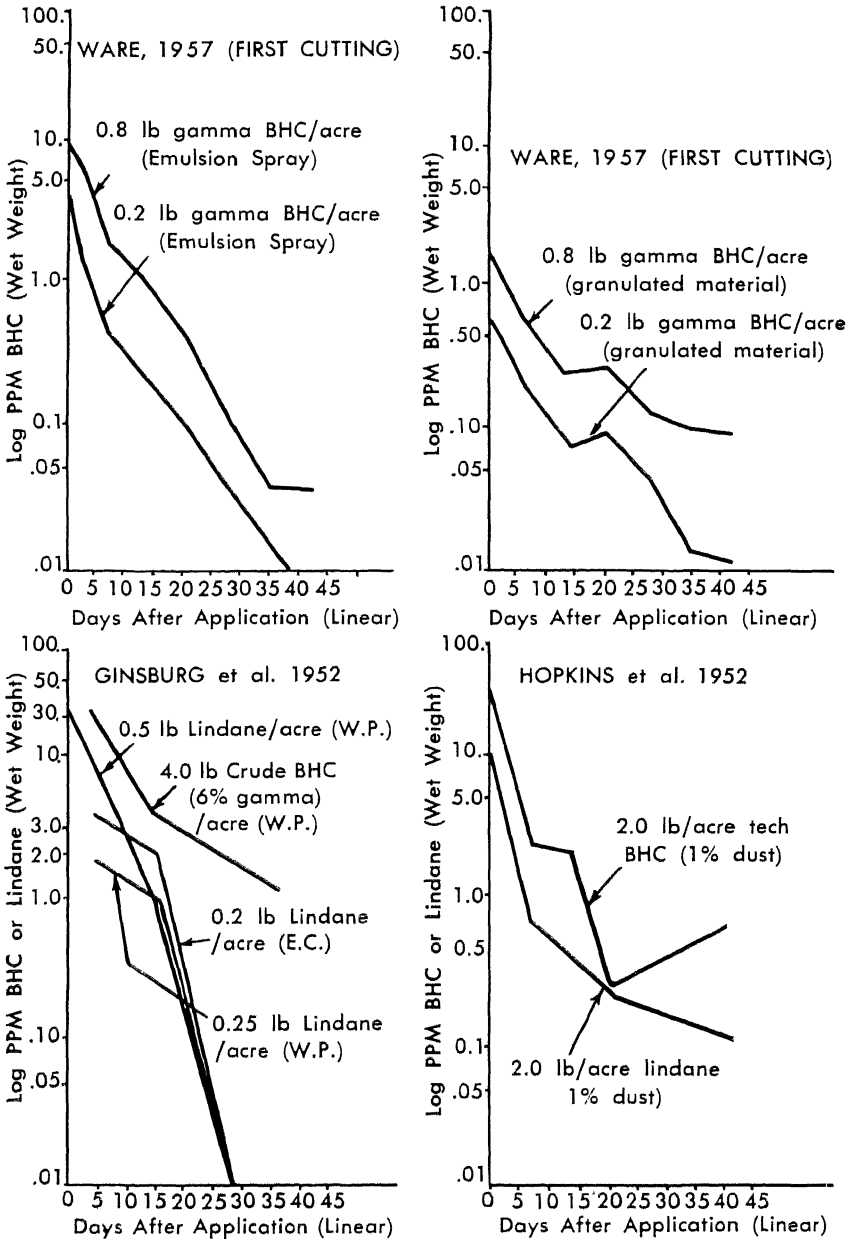

Fig. 6.—Comparison of BHC and lindane residues on alfalfa.

Table 1.—BHC residues on first cutting alfalfa. PPM based on dry weight of samples. Wooster, Ohio, 1957

	Days After Application and Dry Weight of Sample							
	0	3	7	14	21	28	35	42
Formulation and Rate	20.8%	18.3%	15.1%	13.6%	18.1%	17.2%	16.2%*	16.2%*
0.2 pound	2.69	1.67	1.04	0.52	0.45	0.34	0.08	0.06
granular gamma	2.90	2.14	0.97	0.44	0.37	0.19	0.05	0.08
isomer per acre	2.86	2.07	1.40	0.56	0.67	0.24	0.11	----
Average	2.82	1.96	1.14	0.51	0.50	0.26	0.08	0.07
0.2 pound spray	19.49	7.14	2.60	1.25	0.55	0.19	0.06	0.00
(EC) gamma	17.16	6.25	2.47	1.13	0.41	0.07	0.08	0.08
isomer per acre	19.66	5.67	2.90	2.13	0.61	0.34	0.12	----
Average	18.77	6.35	2.66	1.50	0.52	0.20	0.09	0.04
0.8 pound	8.27	5.28	3.08	1.64	1.17	0.67	0.53	0.60
granular gamma	7.58	6.49	3.32	1.40	1.41	0.73	0.56	0.47
isomer per acre	----	5.37	----	2.06	1.60	0.74	0.63	----
Average	7.92	5.71	3.20	1.70	1.39	0.71	0.58	0.54
0.8 pound spray	42.71	----	----	8.11	1.41	0.39	0.07	0.17
(EC) gamma	----	29.29	10.76	7.68	2.30	0.81	0.30	0.25
isomer per acre	49.71	----	13.45	6.34	2.75	----	0.28	----
Average	46.21	29.29	12.11	7.37	2.15	0.60	0.22	0.21

*Dry weights not available, based on average dry weight of other sampling dates.

There are several factors which would prevent the accurate comparison of other workers' results. They are: (1) formulation of BHC, e.g., dust, wettable powder, emulsion sprays, and granulated material; (2) weather conditions, especially immediately following application; (3) a combination of crop age, rainfall and soil type which together would affect the translocation of BHC into the treated crop plant; and (4) wet or dry weight expression of ppm of the toxicant. For convenience to all workers, ppm of the toxicant should be expressed in terms of dry and wet weights of the test plant or plant part (Gunther and Blinn, 1955).

The degradation curves of BHC on alfalfa in this study as well as in others do not conform to a straight line when plotted linearly, semi-log, or log-log. Log ppm versus linear time seemed to produce a near linear relation as seen in Figures 4 and 5.

TABLE 2.—BHC residues on second cutting alfalfa. PPM based on dry weight of samples. Wooster, Ohio, 1957

	Days After Application and Dry Weight of Sample					
	0	2	5	9	14	21
Formulation and Rate	20.9%	16.7%	21.0%	26.9%	23.5%	25.0%
0.2 pound granular	1.95	1.29	0.91	0.31	0.23	0.13
gamma isomer per acre	2.30	1.92	1.26	0.24	0.25	0.35
Average	2.13	1.61	1.08	0.28	0.24	0.24
0.2 pound spray	37.02	30.93	11.81	0.54	1.11	0.29
(EC) gamma	34.34	28.43	10.39	----	1.67	0.41
isomer per acre	64.51	----	----	----	1.18	----
Average	45.29	29.68	11.10	0.54	1.32	0.35
0.8 pound granular	3.63	5.11	3.79	1.49	0.56	0.55
gamma isomer per acre	4.52	2.73	----	1.12	0.72	----
Average	4.08	3.92	3.79	1.30	0.64	0.55
0.8 pound spray (EC)	282.19	151.60	58.45	----	9.30	0.68
gamma isomer per acre	238.56	106.88	78.99	----	6.78	2.42
Average	260.37	129.24	68.72	----	8.04	1.55

This peculiar degradation curve of BHC as compared to some of the other commonly used chlorinated organic insecticides has two probable explanations. Technical grade BHC as it has been commonly marketed varies from 6 to 46 percent gamma isomer (The Pesticide Situation, 1955-56). The BHC available in 1955 until this writing usually contained from 36 to 46 percent of the gamma isomer. Technical BHC is composed of five common isomers, with small quantities of heptachloro- and octochloro-cyclohexanes (Metcalf, 1955). Each of these constituents differs in vapor pressure, boiling point, rate of solubility in organic solvents, and rate of deposition in the fat of mammals (Metcalf, 1955). Technical BHC contains at least six physically different components, of which only the gamma isomer is insecticidal. However, when residue analyses are conducted, only total chlorine or total BHC is used (Phillips, 1952). Both of these systems treat each of the six constituents alike, which in reality is determining the total amount of six different compounds, but referring to them as one. In some respects using either of these methods of chemical determination could be compared to determining total chlorides on a crop which had been treated with a mixture of

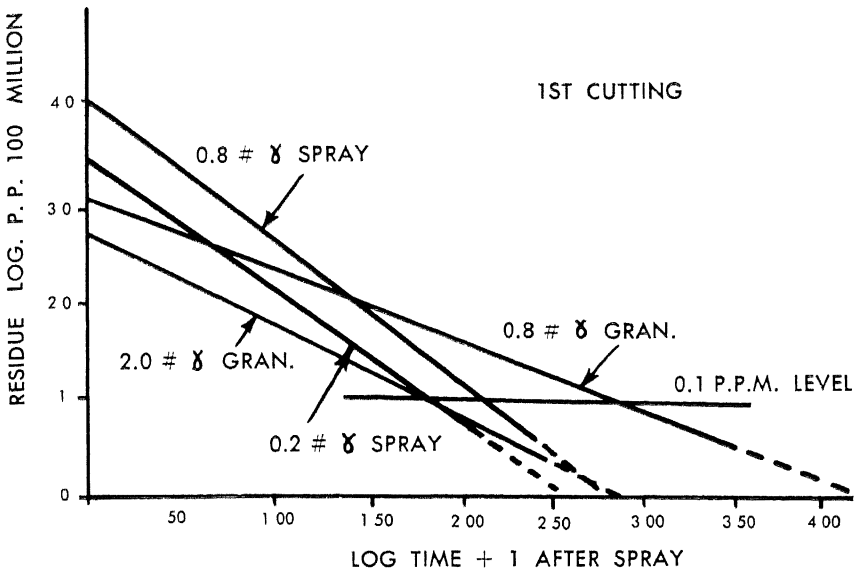

Fig. 7.—BHC residue regression slopes of spray and granular applications on first cutting alfalfa.

aldrin, chlordane, DDT, dieldrin, endrin and heptachlor, and establishing a degradation curve which would be identified as that of ACDDEH, or one insecticide.

The other factor which should have considerable bearing on BHC's persistence is its translocation phenomenon. Work done in this laboratory (Brass, 1958, and Osborn, 1957) and elsewhere (Jameson and Peacock, 1952; Koehler and Gyrisco, 1957; Terriere and Ingalsbe, 1953; and Lichtenstein *et al.*, 1956) indicate that both technical BHC and lindane are translocated in forage and root crops. The regression slopes of granulated and spray applications in Figures 7 and 8 indicate that the granulated applications leave higher residues for longer intervals than do the sprays. To some degree this is borne out in Figure 6 in which the dusts, granulated material, wettable powders, and emulsion sprays are charted. Generally, the dusts, wettable powders and granulated material are more persistent than the emulsion sprays.

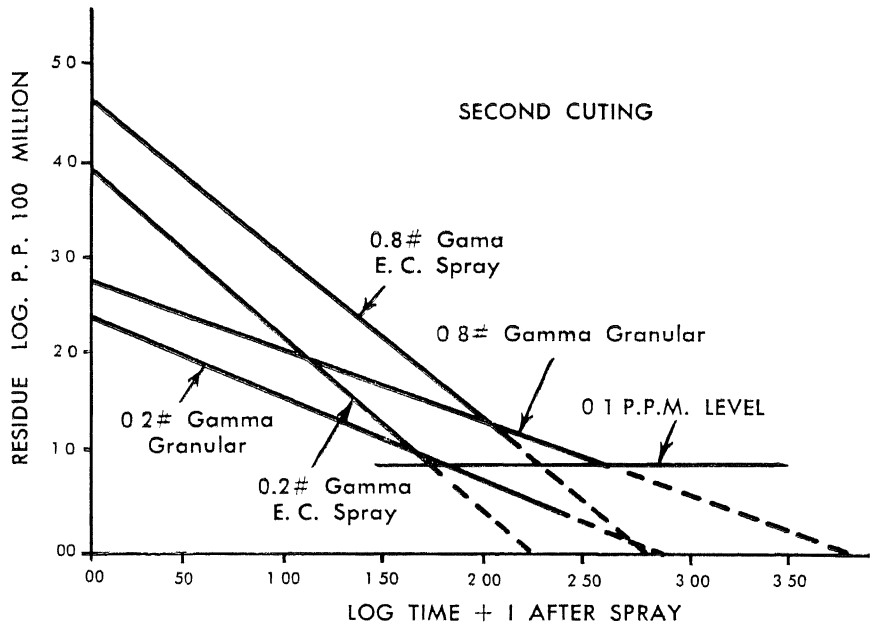

Fig. 8.—BHC residue regression slopes of spray and granular applications on second cutting alfalfa.

As a result of this semi-systemic characteristic of BHC which appears to be more prominent in granulated treatments than in spray or dust applications, the dry weight residues of BHC in first cutting alfalfa 42 days following treatment were 0.07 ppm from 0.2 pound granulated material, and 0.04 ppm from 0.2 pound emulsion spray. These two applications were made at the recommended rate and time for adequate spittlebug control. However, after 42 days, 10 days longer than the crop normally remains in the field, detectable residues were present.

Sternburg and Kearns (1956), using the Schechter-Hornstein colorimetric method, were able to determine quantitatively the presence of a lindane metabolite, pentachlorocyclohexene, in lindane-treated house flies. The metabolite produced a color with maximum absorption at 420 mμ instead of 565 mμ maximum for hexachlorocyclohexane. It could be read independently without interference from the meta-dinitro-

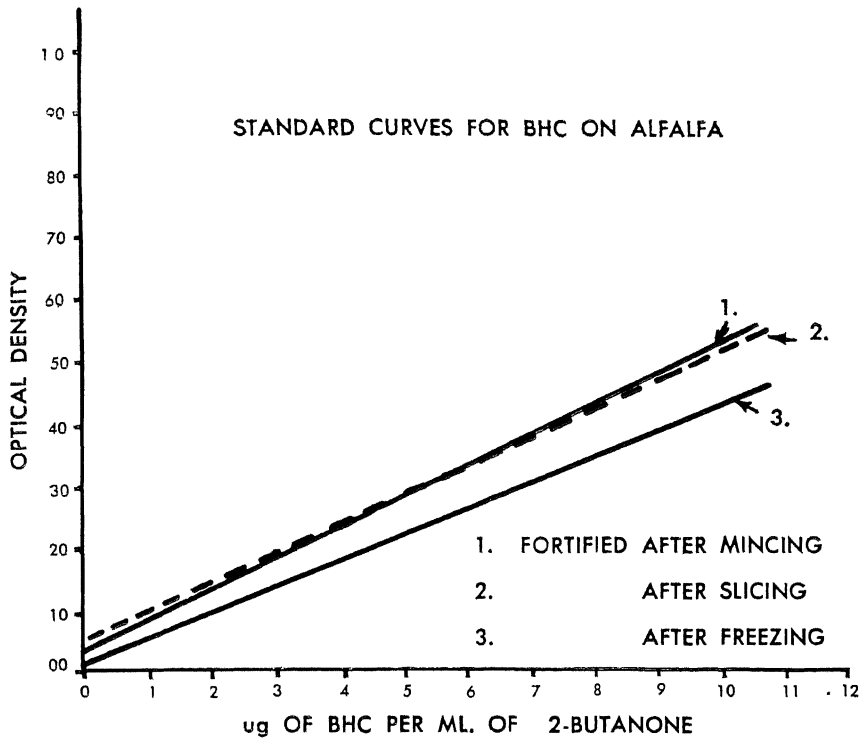

Fig. 9.—BHC standard curves resulting from fortifying at three different stages of extraction.

benzene produced from hexachlorocyclohexane. Bradbury and Standen (1957), however, contended that the pentachloro-metabolite cannot be detected under the conditions prescribed by Sternburg and Kearns.

Considering the possibility that some BHC was metabolized to the pentachloro-form in or on alfalfa, this procedure of Sternburg and Kearns was included in the colorimetric readings. With but few exceptions the readings at 420 mμ for the treated samples were higher than the checks, which were near zero. It is believed from this method of determination that no detectable amounts of pentachlorocyclohexene were present in or on the above-ground portions of the plants when treated with BHC at the rate of 0.2 and 0.8 pound gamma isomer per acre.

SUMMARY

1. Detectable residues of BHC remain on alfalfa beyond normal harvest date when treated with either emulsifiable spray or granlated material at the rate of 0.2 pound gamma isomer per acre.
2. BHC sprays leave higher initial residues on alfalfa than the granulated material when applied at the same rate.
3. Granulated BHC applications resulted in higher residues than sprays 42 days after application on first cutting alfalfa.
4. The degradation curves of BHC sprays are steeper than those of granulated applications on alfalfa.
5. The differences between spray and granulated material degradation curves are attributed to a higher degree of BHC translocation from granule formulations than from sprays.
6. Pentachlorocyclohexene, a possible BHC metabolite, was not detectable on alfalfa at any time following application.

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